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SEQUENCE LISTING

<110> ENDO, Keiji et al.

<120> MUTANT ALPHA-AMYLASES

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<140> US 09/590,375

<141> 2000-06-09

<150> JP P1999-163569

<151> 1999-06-10

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Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys  
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Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn  
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Val Tyr Gly Asp Val Val Met Asn His Lys Met Gly Ala Asp Phe Thr  
100 105 110  
Glu Ala Val Gln Ala Val Gln Val Asn Pro Thr Asn Arg Trp Gln Asp  
115 120 125  
Ile Ser Gly Ala Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Ser  
130 135 140  
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe  
145 150 155 160  
Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Ile Phe Arg  
165 170 175  
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn  
180 185 190  
Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val  
195 200 205  
Gln Asp Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp  
210 215 220  
Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr  
225 230 235 240  
Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu Ala Asp Gln Asp Leu  
245 250 255  
Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe  
260 265 270  
Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu

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Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Met His Ala		
305	310	315
Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu		320
325	330	335
Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu		
340	345	350
Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly		
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Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu		
370	375	380
Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe		
385	390	395
Asp His Trp Asp Val Val Gly Trp Thr Arg Glu Gly Ser Ser Ser Arg		400
405	410	415
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser		
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Lys Trp Met Tyr Val Gly Arg Gln Asn Ala Gly Gln Thr Trp Thr Asp		
435	440	445
Leu Thr Gly Asn Asn Gly Ala Ser Val Thr Ile Asn Gly Asp Gly Trp		
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Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr	50	55	60
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly	65	70	75
Thr Arg Ser Gln Leu Gln Gly Ala Val Thr Ser Leu Lys Asn Asn Gly	85	90	95
Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp	100	105	110
Gly Thr Glu Met Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn	115	120	125
Gln Glu Ile Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp	130	135	140
Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr	145	150	155
His Phe Asp Gly Thr Asp Trp Asp Gln Ser Arg Gln Leu Gln Asn Lys	165	170	175
Ile Tyr Lys Phe Arg Gly Thr Gly Lys Ala Trp Asp Trp Glu Val Asp	180	185	190

Ile Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met  
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 Asp His Pro Glu Val Ile Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr  
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 Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His  
     225                     230                     235                     240  
 Ile Lys Tyr Ser Tyr Thr Arg Asp Trp Leu Thr His Val Arg Asn Thr  
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 Thr Gly Lys Pro Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu  
     260                     265                     270  
 Ala Ala Ile Glu Asn Tyr Leu Asn Lys Thr Ser Trp Asn His Ser Val  
     275                     280                     285  
 Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly  
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 Gly Tyr Phe Asp Met Arg Asn Ile Leu Asn Gly Ser Val Val Gln Lys  
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 His Pro Ile His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro  
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 Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys His Lys Ala Gly  
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 Gln Val Trp Arg Asp Ile Thr Gly Asn Arg Ser Gly Thr Val Thr Ile  
     450                     455                     460  
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ttatgatata tgtaagcggtt atcattaaaa ggaggtatgg atg aga aga tgg gta	176			
Met Arg Arg Trp Val				
-20				
gta gca atg ttg gca gtg tta ttt tta ttt cct tcg gta gta gtt gca	224			
Val Ala Met Leu Ala Val Leu Phe Leu Phe Pro Ser Val Val Val Ala				
-15 -10 -5 -1				
gat gga ttg aac ggt acg atg atg cag tat tat gag tgg cat ttg gaa	272			
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1 5 10 15				
aac gac ggg cag cat ttg aat cgg ttg cac gat gat gcc gca gct ttg	320			
Asn Asp Gly Gln His Trp Asn Arg Leu His Asp Asp Ala Ala Ala Leu				
20 25 30				
agt gat gct ggt att aca gct att ttg att ccg cca gcc tac aaa ggt	368			
Ser Asp Ala Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Tyr Lys Gly				
35 40 45				
aat agt cag gcg gat ttg ggg tac ggt gca tac gat ctt tat gat tta	416			
Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu				
50 55 60				
gga gag ttc aat caa aag ggt act gtt cga acg aaa tac gga act aag	464			
Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys				
65 70 75 80				
gca cag ctt gaa cga gct att ggg tcc ctt aaa tct aat gat atc aat	512			
Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn				
85 90 95				
gta tac gga gat gtc gtg atg aat cat aaa atg gga gct gat ttt acg	560			
Val Tyr Gly Asp Val Val Met Asn His Lys Met Gly Ala Asp Phe Thr				
100 105 110				
gag gca gtg caa gct gtt caa gta aat cca acg aat cgt tgg cag gat	608			
Glu Ala Val Gln Ala Val Gln Val Asn Pro Thr Asn Arg Trp Gln Asp				
115 120 125				
att tca ggt gcc tac acg att gat gcg tgg acg ggt ttc gac ttt tca	656			
Ile Ser Gly Ala Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Ser				
130 135 140				
ggg cgt aac aac gcc tat tca gat ttt aag tgg aga tgg ttc cat ttt	704			
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe				
145 150 155 160				
aat ggt gtt gac tgg gat cag cgc tat caa gaa aat cat att ttc cgc	752			
Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Ile Phe Arg				
165 170 175				

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caa gat gag ttg aag gat tgg ggt agc tgg ttt acc gat gag tta gat Gln Asp Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp 210	215	220	896
ttg gat ggt tat cgt tta gat gct att aaa cat att cca ttc tgg tat Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr 225	230	235	944
aca tct gat tgg gtt cg <sup>g</sup> cat cag cgc aac gaa gca gat caa gat tta Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu Ala Asp Gln Asp Leu 245	250	255	992
ttt gtc gta ggg gaa tat tgg aag gat gac gta ggt gct ctc gaa ttt Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe 260	265	270	1040
tat tta gat gaa atg aat tgg gag atg tct cta ttc gat gtt cca ctt Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu 275	280	285	1088
aat tat aat ttt tac cg <sup>g</sup> gct tca caa caa ggt gga agc tat gat atg Asn Tyr Asn Phe Tyr Arg Ala Ser Gln Gln Gly Ser Tyr Asp Met 290	295	300	1136
cgt aat att tta cga gga tct tta gta gaa gcg cat cc <sup>g</sup> atg cat gca Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Met His Ala 305	310	315	1184
gtt acg ttt gtt gat aat cat gat act cag cca ggg gag tca tta gag Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu 325	330	335	1232
tca tgg gtt gct gat tgg ttt aag cca ctt gct tat gc <sup>g</sup> aca att ttg Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu 340	345	350	1280
acg cgt gaa ggt ggt tat cca aat gta ttt tac ggt gat tac tat ggg Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly 355	360	365	1328
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ctt gat gca cgt caa aat tac gca tat ggc acg cag cat gac tat ttt Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe 385	390	395	1424
			400

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Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser			
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435	440	445	
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465	470	475	480
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Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu			
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Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys			
65 70 75 80			
Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn			
85 90 95			
Val Tyr Gly Asp Val Val Met Asn His Lys Leu Gly Ala Asp Phe Thr			
100 105 110			
Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser Asn Arg Trp Gln Asp			
115 120 125			
Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Pro			
130 135 140			
Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe			
145 150 155 160			
Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Leu Phe Arg			
165 170 175			
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn			
180 185 190			
Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val			
195 200 205			
Gln Glu Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp			

210	215	220
Leu Asp Gly Tyr Arg	Leu Asp Ala Ile Lys His Ile Pro Phe Trp Tyr	
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245	250	255
Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val Gly Ala Leu Glu Phe		
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Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu Phe Asp Val Pro Leu		
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Asn Tyr Asn Phe Tyr Arg Ala Ser Lys Gln Gly Gly Ser Tyr Asp Met		
290	295	300
Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Ile His Ala		
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Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Glu Ser Leu Glu		
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Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu		
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Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly		
355	360	365
Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu		
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Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe		
385	390	395
Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu Gly Thr Ser Ser Arg		
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Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser		
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Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly Gln Thr Trp Thr Asp		
435	440	445
Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile Asn Gly Asp Gly Trp		
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gat ggc ttg aat gga acg atg atg cag tat tat gag tgg cat cta gag   150
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Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys			
65	70	75	80
gct cag ctt gag cga gct ata ggg tcc cta aag tcg aat gat atc aat		390	
Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys Ser Asn Asp Ile Asn			
85	90	95	
gtt tat ggg gat gtc gta atg aat cat aaa tta gga gct gat ttc acg		438	
Val Tyr Gly Asp Val Val Met Asn His Lys Leu Gly Ala Asp Phe Thr			
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Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser Asn Arg Trp Gln Asp			
115	120	125	
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Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp Thr Gly Phe Asp Phe Pro			
130	135	140	
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Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp Arg Trp Phe His Phe			
145	150	155	160
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Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu Asn His Leu Phe Arg			
165	170	175	
ttt gca aat acg aac tgg aac tgg cga gtg gat gaa gag aat ggt aat		678	
Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp Glu Glu Asn Gly Asn			
180	185	190	
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Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe Ser His Pro Glu Val			
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Gln Glu Leu Lys Asp Trp Gly Ser Trp Phe Thr Asp Glu Leu Asp			
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Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala His Pro Ile His Ala			
305	310	315	320

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Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala Tyr Ala Thr Ile Leu	
340 345 350	
acg cgt gaa ggt ggt tat cca aat gta ttt tac ggt gac tac tat ggg	1206
Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr Gly Asp Tyr Tyr Gly	
355 360 365	
att cct aac gat aac att tca gct aag aag gat atg att gat gag ttg	1254
Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp Met Ile Asp Glu Leu	
370 375 380	
cct gat gca cgt caa aat tac gca tat ggc aca caa cat gac tat ttt	1302
Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr Gln His Asp Tyr Phe	
385 390 395 400	
gat cat tgg gat atc gtt gga tgg aca aga gaa ggt aca tcc tca cgt	1350
Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu Gly Thr Ser Ser Arg	
405 410 415	
cct aat tcg ggt ctt gct act att atg tcc aat ggt cct gga gga tca	1398
Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn Gly Pro Gly Gly Ser	
420 425 430	
aaa tgg atg tac gta gga cag caa cat gca gga caa acg tgg aca gat	1446
Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly Gln Thr Trp Thr Asp	
435 440 445	
tta act ggc aat cac gcg gcg tcg gtt acg att aat ggt gat ggc tgg	1494
Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile Asn Gly Asp Gly Trp	
450 455 460	
ggc gaa ttc ttt aca aat gga gga tct gta tcc gtg tat gtg aac caa	1542
Gly Glu Phe Phe Thr Asn Gly Gly Ser Val Ser Val Tyr Val Asn Gln	
465 470 475 480	
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<210>13  
<211>25  
<212>DNA  
<213>Artificial Sequence

<220>  
<223> PCR Primer

<400>13  
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<210>14  
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<220>  
<223> PCR Primer

<400>14  
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<211>33  
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<220>  
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<210>16  
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<220>  
<223> PCR Primer

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<223> PCR Primer

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<223> PCR Primer

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<223> PCR Primer

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<212>DNA  
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<220>

<223> PCR Primer

<400>22  
taaagcttca atttatattg g 21

<210>23  
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<223> PCR Primer

<400>23

ccagatctac ttaccattt agagtca 27

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